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Solvent-Free Plasticity and Programmable Mechanical Behaviors of Engineered Proteins

Chao Ma, Juanjuan Su, Bo Li, Andreas Herrmann, Hongjie Zhang, and Kai Liu*

Biopolymeric networks with plasticity show great competences in diverse fields owing to the combined biocompatible and mechanical characteristics. However, to realize such plasticity external complicated treatments, e.g., UV or organic solvent have to be applied, which in turn impair the biological nature and even mechanical properties of those systems. To address this challenge, one new type of anhydrous protein liquid crystalline (LC) gels, which exhibit flexible morphological plasticity and mechanical programmability is demonstrated. Supramolecular interactions in the smectic biogels play an important role for their high plasticity. Remarkably, the samples exhibit outstanding mechanical behaviors. The tensile strength and Young's modulus at MPa levels are comparable or even higher than chemically cross-linked hydrogels and LC elastomers. More importantly, mechanical programmability of the LC gels is achieved by genetically tuning the charge density of protein backbones. Consequently, the mechanical performance is manipulated in the range of one order of magnitude. Thus, this type of anhydrous protein LC gels offers great opportunities for load-bearing high-tech applications.

The realization of plasticity of structural biomaterials is an important step in the contexts of engineering, applied science, and biomedicine due to anisotropic nature and ease of

manipulation in the systems.^[1–7] Typically, external stimuli to actively trigger such plasticity include optical-,^[8,9] electrical-,^[10] thermo-^[11] as well as solvent-treatments.^[12,13] However, in biological systems, the applied inputs such as ultraviolet light, high temperature or organic solvents involved in the responsive processes may significantly destabilize sophisticated structures of biopolymers (e.g., helix of DNA or folding of proteins), leading to the impaired mechanical behaviors of biomaterials.^[14] Thus, it is challenging to realize high plasticity in biomacromolecular materials with preserved structural stability and functional integrity.

Biopolymeric soft materials have notable competences in material engineering because of ingenious structure, biocompatibility, and facile bioconjugation.^[15–17] For instance, this class of materials can be developed as bone matrix/

scaffold in the context of tissue engineering^[18–22] and mechanically robust devices for biomedical implant.^[23–25] Yet, to present plasticity behavior in those systems, covalent crosslink agents and/or solvent exposure are prerequisite treatment, which in turn damage the output mechanical performance of the materials, limiting their technical applications.^[26,27] Therefore, to avoid the shortcomings, it is highly desirable to assemble new generation of biomacromolecular soft materials.

Herein, we demonstrate an anhydrous biological liquid crystalline (LC) gel with great plasticity, which is realized by assembly of super-negatively charged proteins and cationic surfactant. The multiple supramolecular interactions involving electrostatic forces, hydrogen bonding, and van der Waals interactions afford a type of persistent and adaptive noncovalent anisotropic network, offering extraordinary properties including facile deformation and robust mechanical performance. The anhydrous nature in the gel also allows for stress-induced plasticity. The formed LC fibers exhibit strong performance, which is comparable or even higher than chemically crosslinked soft materials, e.g., hydrogels and LC elastomers. More importantly, by taking advantage of molecular cloning technique, the length and charge density of molecular backbone of the protein were precisely controlled, allowing for programmability of the materials' mechanical performance. Thus, this class of protein LC gels host great promise for mechanical-related biomedical applications.

The proteins employed in this study are derived from elastin polypeptides, a major type of proteinaceous components in

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Figure 1. Fabrication and plasticity of the solvent-free protein LC gels. a) Schematic illustration on the production of negatively charged proteins. Customized plasmids were designed and synthesized. The *E. coli* cells worked as a bioreactor to express the proteins with high fidelity. Two types of proteins were collected, including the (VPGEG)_n and double-charged series (VEGEG)_n. The gray box in the schematic figure at gene level indicates a promoter and the red box represents a terminator. b) Self-assembly of the proteins and didcylidimethylammonium (DEAB) surfactant for the formation of LC gels. c) The LC gels showed shape deformation and flexibility. d) Continuous LC fibers with homogenous nature were easily drawn from the gel matrix, suggesting high plasticity of the engineered protein LC network. e) The stretched protein LC fibers with ≈10 cm length.

extracellular matrix. The unstructured elastin molecule consists of repetitive pentapeptide units, carrying valine, proline and glycine residues. We employed molecular cloning to specifically introduce glutamic acid into the fourth position of the pentapeptide unit, thus yielding negatively charged protein (VPGEG)_n with a charge in every five amino acid residues, termed E series herein. To get a higher charge density, a second glutamic acid residue was inserted into the sequence, resulting in double charged EE series (VEGEG)_n with one charge per 2.5 amino acids. The genetic engineering of customized plasmids and downstream protein expression were performed using *Escherichia coli* cells (Figure 1a and Figure S1, Supporting Information). Negatively charged E36, E72, E144 and double charged EE36, EE54, EE108 were produced for further analysis. Notably, the E36, (VPGEG)₃₆, indicates that there are 36 charges embedded in the polypeptide backbone with 36 repeating units. The double charged EE series, such as EE36, (VEGEG)₁₈, represents totally 36 charges in 18 repetitive units. Furthermore, a cysteine residue is introduced at the C-terminus of EE series to yield disulfide bonds between polypeptide chains. The full sets of molecular details, particularly amino acid compositions of all samples, are shown in Table S1 (Supporting Information).

The negatively charged proteins were then electrostatically complexed with cation surfactant of didcylidimethylammonium bromide (DEAB), leading to gel-like precipitation from the aqueous solution. Through lyophilized dehydration process a solvent-free gel state was realized (Figure 1b,c). Thermogravimetric analysis (TGA, Figure S3, Supporting Information) indicated that there was only trace-amount water (less than 5%, wt%) in the gel complex. The gel showed flexibility with shape deformation and could be easily molded into round or irregular contour (Figure 1c). More importantly, this class of gel materials have excellent stress-induced plasticity behavior. Bulk soft fibers with homogeneous diameter ($\varnothing \approx 200 \mu\text{m}$) were readily

drawn from the supramolecular self-assembled LC gel samples (Figure 1d,e).

Polarized optical microscopy (POM) was utilized for the characterization of specific patterns of the mesophases assemblies (Figure 2a). Here EE36-DEAB complex was shown as an example. Focal-conic texture was detected, which is a characteristic smectic structure of LC gel materials. The complex was further analyzed with differential scanning calorimetry (DSC) presenting a thermo-profile investigation. The endothermic peak at 8 °C in the DSC curve was ascribed to the phase transition of the complex from crystallized state to LC gel state, and the temperature at 85 °C corresponded to the transition of LC gel-isotropic gel state (Figure S4, Supporting Information). Notably, the exothermic peak at −37 °C was due to the crystallization of the LC gel material. When applied with external tensile force, the complex exhibited high plasticity and it easily underwent a deformation, yielding a homogeneous LC fiber (Figure 2b). Intense birefringence of the soft fiber was obtained by POM (Figure 2c), implying that the molecular alignment and smectic orientation in the fiber network became uniform.

Next, we applied small angle X-ray scattering (SAXS) to validate the structure of EE36-DEAB gel complex. The sharp first-order reflection peak is the characteristic signal of long-range ordered lamellar structures with ≈2.46 nm spacing (Figure 2d). A 3D model of the complex structure is proposed based on the X-ray data (Figure 2e). The protein sublayer is ≈1.0 nm thickness shown in red and cyan color. At the same time, surfactants self-assemble into alternate repeating sublayers showing 1.46 nm distance presented in green. The tail-to-tail packed surfactant layers actively interact with the negatively charged unstructured protein layers via supramolecular interactions, thus leading to internal smectic LC ordering in the system. The periphery of each polypeptide is decorated by alkyl chains. Since hydrophobic interactions of aliphatic chains in surfactant is important for supramolecular gelation, the interactions

Figure 2. Characterization of the mesophases and structure details of the protein LC gels and the formed LC fibers. a) Typical focal-conic texture of the LC gel was visualized under polarized optical microscopy (POM). b) A photo of the as-spun LC fibers. c) The fiber was imaged using POM and exhibited strong birefringence behavior, indicating uniform orientation of the protein-surfactant complex. d) SAXS analysis of the gel LC mesophase. A characteristic 1st order reflection peak ($q_1 = 0.255 \text{ \AA}^{-1}$) of EE36-DEAB complex was collected, indicating a lamellar-spacing structure. The broad peak labeled with an asterisk ($q_2 = 0.4 \text{ \AA}^{-1}$) is the Kapton signal. e) A side view of 3D model exhibits smectic packing of the protein-surfactant complex. The average distance is 2.46 nm, indicating the spacing of aliphatic hydrocarbon surfactant sublayer (green, $\approx 1.46 \text{ nm}$) and protein sublayer (red and cyan, $\approx 1 \text{ nm}$). The periphery of each polypeptide is decorated by alkyl chains. The van der Waal forces and hydrophobic interactions between alkyl tails are important for supramolecular gelation of the protein-surfactant complexes and the final formation of a solvent-free LC gel network.

between alkyl tails might be the driving force for the gelation of the protein-surfactant complexes and the final formation of a solvent-free LC gel network.

To investigate the viscoelastic behavior of the solvent-free LC gel materials, rheology measurements were performed (Figure 3a). The modulus data (storage part G' and loss part G'') were collected at a fixed strain amplitude of 10%. A temperature-modulus profile of the complex was studied. For the EE54-DEAB complex, at room temperature ($T = 25 \text{ }^\circ\text{C}$) over the applied frequency range, the G' reached to a plateau with peak value of 0.5 MPa, far beyond the G'' (less than 0.05 MPa), suggesting a robust elasticity of this protein LC gel. When heating to $40 \text{ }^\circ\text{C}$, G'' was initially a little bit larger than G' , yet after a relaxation time, the G' became greater than G'' at a crossover frequency of 15 Hz, indicating the recovered elastic gel state of the complex. Heating further to $60 \text{ }^\circ\text{C}$, the increase in loss modulus resulted in G'' higher than G' although the material is still in the smectic mesophase (Figure S5, Supporting Information). At $90 \text{ }^\circ\text{C}$ which is above the transitional clearing point, both values of G' and G'' became trivial, probably due to the isotropic state of the gel network. In addition, we found that the viscosity reduced significantly along with the temperature increasing, which is coincident with the gel phase transition from the smectic to isotropic phase (Figure 3b). Interestingly, the double charged protein complex showed stronger mechanical performance than the single charged material complex. Specifically, with the same backbone length, the viscosity of double charged series is higher than the single charged ones (Figure 3c). The storage modulus of EE series is also stronger

than E series (Figure 3d). For example, the monomer of EE36 has 18 repeat units. There is only one cysteine residue at each C-terminus of the polypeptide chain. Therefore, if complete dimerization resulted EE36 dimer has 36 repeat units and 72 charges. E36 also has 36 repeat units but only 36 charges. However, the storage moduli of E36 gel (0.1 MPa) is much smaller than that of EE36 gel (0.33 MPa). In our preparation condition, there is estimated $\approx 50\%$ dimerization. Therefore, the stronger mechanical performance of EE36-DEAB than E36-DEAB samples can be due to the increased packing amount and density of complexed surfactant. This leads to increased intermolecular interactions in the gelation process of supramolecular assembly. This also holds true for the comparison of EE54-DEAB LC gel and E72-DEAB counterpart. Thus, those data exclusively suggest that the mechanical performance of the LC gels can be programmed via charge density of the engineered proteins.

Next, we characterized the mechanical behaviors of the protein LC gels by tensile testing of the plasticized fibers (Figure 4a). Stress-strain plotting profiles were recorded (Figure 4b,c). The tensile strengths of E series LC fibers varied from 30 to 100 KPa and further to 390 KPa, along with the increasing of molecular backbones of E series proteins from E36 repetitive units to 72 units and to 144 repeats. Their Young's moduli accordingly changed from 400–500 to 2400 KPa. By doubling the charge density of the engineered proteins, it showed that both breaking strengths and moduli were remarkably increased (Figure 4c–e). The statistically average strength of EE108 fibers reached 500 KPa and the modulus was up to 3000 KPa, although the

Figure 3. Rheology study of the solvent-free protein LC gels. a) A systematic investigation of storage (G') and loss (G'') moduli of double-charged EE54-DEAB complex with varied temperatures. Both storage and loss modulus were recorded over a course of applied frequency. The material showed robust mechanics at room temperature condition, presenting high storage modulus (>0.5 MPa). With increased temperature, both moduli decreased substantially. b) Viscosity behavior of the EE54-DEAB gel was studied along with increased temperature profile. The viscosity of the system decreased when the material was thermally treated. c) Viscosity investigation of the single-charged and double-charged protein LC system showed larger values of viscosity. d) Storage modulus investigation of the single-charged and double-charged protein LC systems. The complexes prepared with EE series exhibited stronger mechanical strength even at lower repeating units.

molecular weight of EE108 is even smaller than that of E144. Consequently, both tensile strengths and Young's moduli of the protein gels were manipulated conveniently in the range of one order of magnitude. The maximum elongation of EE gel fibers was also greater than E series, resulting in a tougher gel system (Table S2, Supporting Information). Therefore, the data suggest that the mechanical performance is positively associated with the charge density of the proteins.

Moreover, it is found that the EE LC gels generally have a stronger tensile strength, Young's modulus, and toughness than the E gel series by spider chart analysis (Figure 4f). Thus, it suggested that by modulating the charge density of proteins via molecular cloning, the amounts of surfactants embedded in the system can be adjusted, leading to programmable mechanical properties of the bulk biomaterials. This means that the output performance of this class of LC gel materials can be simply designed via initial inputs at the genetic level. Moreover, the mechanical properties of the EE LC gel samples, especially in terms of strength and elastic modulus, are comparable or even higher than those of chemically covalent cross-linked hydrogels and LC soft matters (Table S3, Supporting Information).^[28–31] The network formed by the interactions of the alkyl chains is very different from conventional networks and contributes to the extraordinary mechanical behaviors. Because of the internal smectic ordering in the new gel system, this structure composed of protein sublayer and intercalated surfactant

sublayer is totally regarded as a rigid part and responsible for the high strength of the LC gel networks. When it is deformed, the long-ranged sliding of alkyl chains within the sublayers of surfactants and the van der Waal forces and hydrophobic interactions between alkyl chains which could easily rebuild between neighboring sublayers endow the bulk material with good extensibility and plasticity.

In summary, we prepared a family of anhydrous protein LC gel assemblies driven by electrostatic interactions. The smectic structure and multiple supramolecular interactions endow this class of LC gels with robust mechanical performance and great plasticity. Moreover, by tuning the charge density of proteins, the tensile strength and elastic modulus can be actively programmed in the range of one order of magnitude. The long-range aligned lamellar structure dictates the high mechanical performance of the protein gels at bulk scale. In stark contrast to traditional covalent crosslinked systems, here supramolecular interactions of the unstructured protein backbones and surfactants play a vital role for the gels' formation and high plasticity. This type of driving forces also made the mechanical performance of the protein gels comparable or even higher than the chemically crosslinked hydrogels and LC elastomers. The recombinant cloning approach to tune the materials mechanics is simple and easy to handle. Therefore, this strategy greatly broadens the design and fabrication of mechanical biomaterials with programmable properties. Further, this new generation of

Figure 4. Programmable mechanical behaviors of the protein LC gel fibers. a) A photograph of the tensile test device loaded with the LC gel fiber. b) Stress–strain curves of a series of single charged E-DEAB LC gels. Here E36, E72, and E144 were employed to fabricate the single charged gels. c) Stress–strain curves of a series of double charged EE-DEAB LC gels. Here EE36, EE54, and EE108 were employed to fabricate the double charged gels. d,e) The corresponding tensile strengths and Young's moduli of the protein LC gel fibers. In comparison of the two types of the LC gels, the EE LC gel fibers exhibited stronger tensile strengths and Young's moduli than the E LC gel fibers. Mechanical performance of the solvent-free protein LC gels was manipulated in the range of one order of magnitude. This indicates that programmable mechanical properties of the bulk biomaterials can be realized by genetically tuning the charge density of the engineered proteins. f) Spider chart representing the mechanical performance of the protein LC gel fibers. The EE LC gels generally have stronger tensile strength, Young's modulus, and toughness than the E LC gels.

protein LC gels showing extraordinary mechanical properties and plasticity will have applications in the contexts of biomechanics and biomedicine where both noncovalent interactions and robust mechanics are preferred.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

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